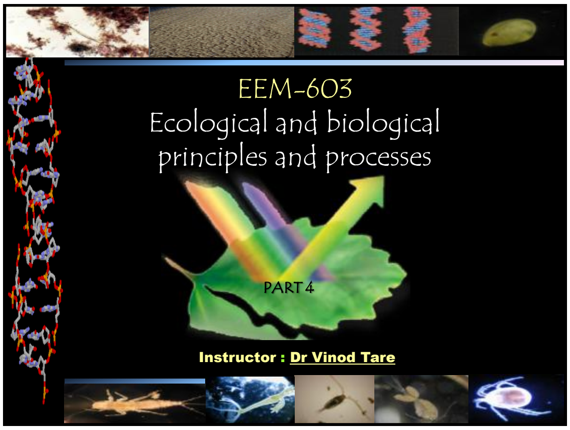


Microbial World and Microbiology

- Look in any direction, and you will see signs of microorganisms at work
- Bacteria help some plants grow by capturing nitrogen from the air
- Bacteria and fungi degrade waste such as dead plants, oil from spills, sewage, and discarded food
- Food production, drug manufacturing, and other industries frequently utilize microorganisms or their by-products
- Found nearly everywhere, microorganisms are the most widely distributed group of organisms on earth
- You are a home to roughly 100 trillion microorganisms
- MOs are on your skin and hair, in the tartar on your teeth, along your intestine, and elsewhere on body surfaces

Microbial World and Microbiology

- Every gram of waste material your body discharges from the large intestine contains 10 billion microorganisms, which are quickly replaced by others
- No other organisms have the ability to chemically alter substances in as many ways as do microorganisms
- Chemical changes caused by microorganisms are called biochemical changes, because they involve living organisms
- Some of these biochemical reactions are the same as those in other forms of life, including humans
- Such similarities, coupled with the convenience of studying microbes, make these organisms important in research
- Chemists, physiologists, geneticists, and other frequently use microbes to explore the fundamental process of life

Microbiology

- Concerned with all aspects of microorganisms
  - Structure; nutrition; reproduction; heredity; chemical activities; classification and identification
  - Distribution and activities in nature; relationship to each other and to other organisms, and ability to cause physical and chemical changes in the environment
  - How the microorganisms affect the health and welfare of all life on the earth.

Basic and Applied Microbiology

Cells and Cell Theory

- The word *cell* first appeared in 1665, when an Englishman, Robert Hooke, used it to describe plant materials he saw through his microscope → looking at thin slice of cork, he noted the honeycomb like structures formed by the walls of once-living cells.
- On this basis and other observations, two German scientists (*Matthias Schleiden and Theodore Schwann*) developed the *cell theory* in 1938-39.
- They suggested that cells are the basic structural and functional units of all organisms
  - From single celled microorganisms (MO) to life forms with specialized tissues and complex organ system
- As the cell theory gained acceptance, investigators speculated about the substance within the cell, *protoplasm* (the "first formed substance").

Protoplasm

Is a complex, gelatinous mixture of water and organics (carbohydrates, proteins, lipids and nucleic acids), enclosed by flexible membrane and sometime by rigid cell wall.

Nucleus

- Region that controls cell function and inheritance
- Contains coded information
- Surrounded by membrane (in some cells), nuclear membrane
- Nucleoid, no membrane (in some cells)

Cytoplasm

- Non nuclear area remainder of protoplasm

All the life processes take place within the cell in a unicellular organism, while in multicellular organism each cell/tissue/organism performs a specific function

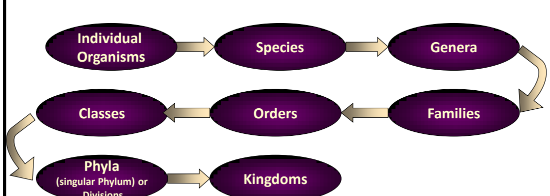
Organisms' Basic Characteristics

- Reproduce
- Use food as source of energy
- Synthesize cell substance and structures
- Excrete wastes
- Respond to change in the environment
- Mutate, through infrequent, sudden changes in their hereditary characteristics.

Classification of Living Organisms

- There are about 10 million species of living organisms in the world, including thousands of microbial species
- The need to make order out of this great number and variety of organisms is characteristics of human mind
- Placed into groups based on their similarities
- The science of taxonomy includes classification (arrangement), nomenclature (naming), and identification (description and characterization) of living organisms
- Organisms that share certain common characteristics are placed into taxonomic groups called texa (singular taxon)
- The basic Taxon is the species

Classification of Living Organisms



Some Examples of the Classification of Organisms

	Organism		
Taxa (categories)	Cat	Alga	Bacterium
Kingdom or Major Group	Animal	Plant	Eubacteria
Division		Chlorophyta	Gracilicutes
Phylum	Chordata		
Subphylum	Vertibrata		
Class	Mammalia	Chlorophyceae	Scotobacteria
Subclass	Eutheria		
Order	Carnivora	Volvocales	Spirochaetales
Family	Felidae	Chlamydomonadaceae	Leptospiraceae
Genus	Felis	Chlamydomonas	Liptospira
Species	*F. domesticus*	*C. eugametos*	*L. interrogans*

Species

- Collection of strains with similar characteristics in their hereditary material (a strain is made up of the descendants of a single colony from a pure culture)
- Features used to place organisms into species include morphology and nutritional requirements

Genera (singular genus)

Group of closely related species

Classification of Living Organisms

- Name of the species is always given as a two-part Latin combination (binomial) → Consisting of the genus name and a specific name that denotes the species.
  - *Homo sapiens* or *H. sapiens*
  - *Escharichia coli* or *E. coli*
- Because of different traditions among the various biological sciences, there is no consensus on the nomenclature and classification of every taxon.
- Zoologists and botanists, agree with few exceptions, on the general arrangement of animals and plants into phyla/divisions but microbiologists have not established phyla that satisfy bacteriologists, phycologists, protozoologists, and others.
  - Partly because of this lack of agreement, the genus and the species remain the two most important texa among bacteria.

Classification of Living Organisms

- During mid-eighteenth century, Carolus Linneeus developed the binomial system and placed all living organisms in two kingdoms, Plantae or Animalia.
  - Pioneering work, great scientific work, however, classification systems were misleading or just plain wrong because they were based on inaccurate information.
- Classification systems, particularly those for MO's are still evolving as more discoveries are made about physical and chemical characteristics.
- Two kingdom classification for MO's → Protozoa in animal & other MO's in Plants.
  - Concept is impractical, particularly for microorganisms, as some have characteristics of both, plant and animal.

Classification of Living Organisms

- In 1866 Ernst H Haeckel (Student of Charles Darwin) proposed a third kingdom called Protista for those MO's who have features of both plants and animals, and included bacteria, algae, yeasts, and protozoa in this.
  - ➢ But validity of this kingdom is questioned as more information about the internal structures of microbes became available.

Procaryotic & Eucaryotic Microorganisms

- Based on how nuclear substance exists within the cell (An important discovery in terms of taxonomy for microbial cells based on advances in electron microscopy).
  - Bacteria are procayrotes (major criteria for separation from other microbes)
  - Algae, fungi, protozoa and cells of plants and animals have eucaryotic cell structure and are eucoryotes.

The Five-Kingdom Concept of Classification

- The Five-Kingdom Concept Based on Obtaining Nutrition from Food (By Whittaker in 1969): Three levels of cellular organization have evolved to accommodate three principle modes of nutrition.
  - Photosynthesis (light energy to convert CO_2 to sugars)
  - Absorption (uptake of chemical nutrients dissolved in water)
  - Ingestion (intake of insoluble particles of food)

The Five-Kingdom Concept of Classification

1. **Monera** (prokaryotes → bacteria): Considered the most primitive kingdom and thought to be the ancestors of the eucaryotes.
2. **Protista** (Eucaryotes → unicellular MO's, principally algae and protozoa, and also the slime molds, the lower fungi) → Represent all three categories of food intake. (Algae → photosynthesis; Protozoa → ingest; Slime molds → only absorb)
3. **Plantae** → higher eucaryotic organisms which are photosynthetic → green plants & higher algae.
4. **Animalia** → Animals which ingest food.
5. **Fungi** → organisms that have cell walls but lack the photosynthetic pigment chlorophyll found in other plants and thus absorb their food.

Archaeobacteria, Eubacteria, and Eucaryotes

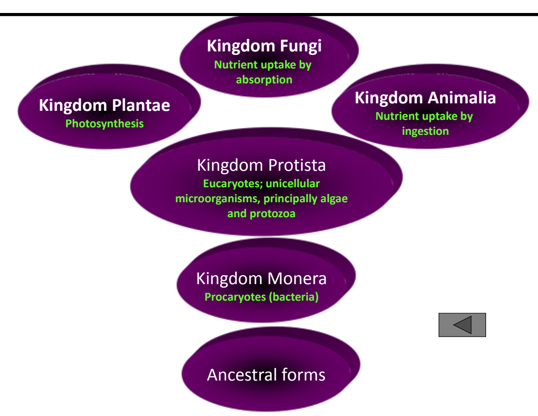
- Monera & Protista → considered as ancestral forms; pro → earlier than; → Different ancestral pattern, established now based on rRNA.
- Archaeobacteria, eubacteria, and eucaryotes evolved through separate pathways from a common ancestor.
- The Endosymbiotic Theory

Major Schemes of Classification of Living Organisms

Classification scheme	Kingdoms	Organisms included
Linnaeus (1753)	Plantae	Bacteria, fungi, algae, plants
	Animalia	Protozoa and higher animals
Haeckel (1865)	Plantae	Multicellular algae and plants
	Animalia	Animals
	Protista	Microorganisms, including bacteria, protozoa, algae, molds, and yeasts
Whittaker (1969)	Plantae	Multicellular algae and plants
	Animalia	Animals
	Protista	Protozoa and single-celled algae
	Fungi	Molds and Yeasts
	Monera	All bacteria (procaryotes)

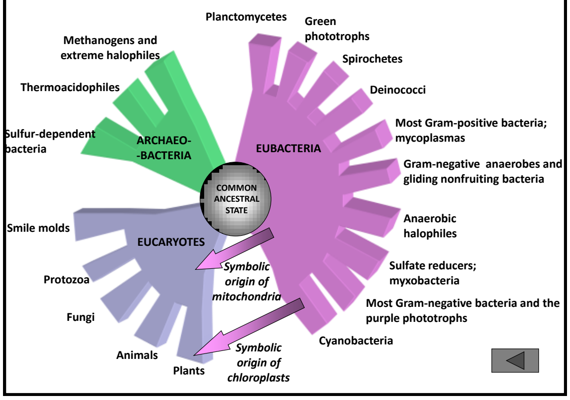
Major Schemes of Classification of Living Organisms

Classification scheme	Kingdoms	Organisms included
Woese (1977)	Archaeobacteria	Bacteria that produce methane gas, require very high levels of salt, or require very high temperatures
	Eubacteria	All other bacteria, including those most familiar to microbiologists, such as disease causing bacteria, photosynthesic bacteria
	Eucaryotes	Protozoa, algae, fungi, plants, and animals



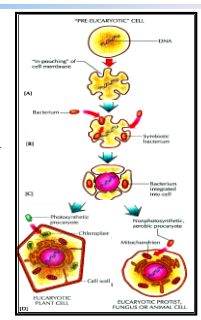
Simplified scheme of Whittaker's five-kingdom classification of living organisms

A depiction of the pathways by which living organisms evolved, as deduced from comparative studies of ribosomal RNA. The three major evolutionary branches are shown leading to present-day archaeobacteria, eubacteria, and eucaryotes. Within the eubacterial branch, at least 10 distinct lines of descent occurred; in the archaeobacteria branch, at least three distinct lines of descent occurred. In the case of eucaryotes, there is evidence that certain Gram-negative eubacteria invaded a primitive from of eucaryotic cell and evolved as specialized intracellular organelles called *mitochondria*. Chloroplast, the photosynthetic organilles of plant cells, appear to have evolved in a similar manner from cynobacteria.



The Endosymbiotic Theory

The endosymbiotic theory, which proposes the manner in which eucaryotic cells may have evolved. This theory suggests that a "pre-eucaryotic" cell developed an "in-pouching" of the cell membrane [A]. Bacteria entered the "in-pouched" area as symbionts [B] and became an integral part of the cell [C]. When the bacterial symbiont was a photosynthetic procaryote, it functioned as a chloroplast and a plant cell evolved. When the bacterial symbiont was a nonphotosynthetic aerobe, it functioned as a mitochondrion (providing energy) and an animal or protist type of cell evilved [D].



Some Differential Characteristics of Procaryotes and Eucaryotes

Characteristic	Procaryotes	Eucaryotes
Genetic material seperated from cytoplasm by a membrane system	No	Yes
Usual cell width or diameter	0.2 to 2.0 µm	>2.0 µm
Mitochondria	Absent	Present
Chloroplast (in photosynthetic species)	Absent	Present
Endoplasmic reticulum and Golgi complex	Absent	Present
Gas vacuoles	Formed by some species	Absent
Poly-β-hydroxylbutyrate inclusions	Formed by some species	Absent
Cytoplasmic streaming	Absent	Often Present
Ability to ingest insoluble food particles	Absent	Present in some species
Flagella, if present:		
▪ Diameter	0.01 to 0.02 µm	Ca. 0.2 µm
▪ Cross section shows "9+2" arrangement of microtubules	No	Yes

Some Differential Characteristics of Procaryotes and Eucaryotes Contd….

Characteristic	Procaryotes	Eucaryotes
Heat resistant spores (endospores)	Formed by some species	Absent
Polyunsaturated fatty acids or sterols in membranes	Rare	Common
Muramic acid in cell well	Common	Absent
Ability to use inorganic compounds as a sole energy source	Present in some species	Absent
Ability to fix atmospheric nitrogen	Present in some species	Absent
Ability to dissimilate nitrates to nitrogen gas	Present in some species	Absent
Ability to produce methane gas	Present in some species	Absent
Site of photosynthesis, if it occurs	Cytoplasmic membrane extensions; thylakoids	Garna of chloroplasts
Cell division occurs by mitosis	No	Yes
Mechanisms of Gene transfer and recombination, if they occur, involve gametogenesis and zygote formation	No	Yes

Some Differential Characteristics of Procaryotes and Eucaryotes Contd….

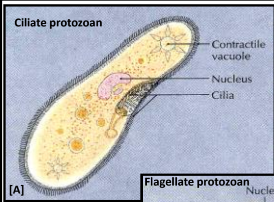
Characteristic	Procaryotes	Eucaryotes
Chromosomes:		
▪ Shape	Circular	Linear
▪Number per cell	Usually 1	Usually > 1
Ribosomes:		
Location in cell	Dispersed throughout cytoplasm	Attached to endoplasmic recticulum
Sedimentation constant (in Svedberg units)	70 S	80 S*

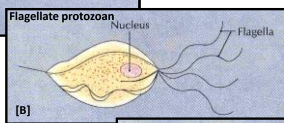
* Except in mitochondria and chloroplast, which have ribosomes of the procaryotic type (70 S)

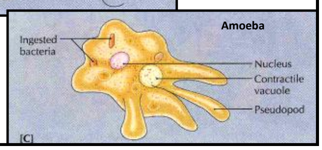
The Major Groups of Organisms Based on Certain Traits (Characteristics or Actions)

Protozoa, Fungi, Algae, Bacteria; and Viruses.?

- **Protozoa:**
  - Single celled, Eucaryotic MOs
  - Animal like (ingest food), lack of rigid cell wall. Do not contain chlorophyll.
  - Some can swim through the beating action of short hair like appendages called flagella.
  - Other protozoa, do not swim but can creep along surfaces (amoebas).
  - Other type, called sporozoans **>** they form resting bodies, called spores during one phase of their life cycle **>** usually non-mobile in this phase
  - Occur widely (in nature)
  - Some cause animal and human diseases
  - Some are beneficial, help digesting the food in some animals.







Schematic illustration of several types of protozoan cells

Protozoa are microscopic organisms with animal like characteristics.

Algae

- Considered plant like, contain green pigment chlorophyll, carry out photosynthesis, have rigid cell walls.
- These are eucaryotes, may be unicellular and microscopic or multicellular and upto several meters in length.
- Problems: clogging, releasing toxics, growing in swimming pools, etc.
- Uses (extracts from some algae): Thickeners and emulsifiers for foods such as ice creams, custards, as an anti inflammatory drugs for ulcer, as a source of agar.

Fungi

- Eucaryotic like algae (single or multicellular) (mushrooms and bracket fungi growing on damp layers of soil)
- Do not contain chlorophyll --- can not carry out photosynthesis.
- Absorb dissolved nutrients.
- Fungi (which are MOs) with multicells, producing filamentous microscopic structures are called molds > Cells are cylindrical in shape and are attached end to end, thread like called hyphae >Individually hyphae are microscopic in size > When large number accumulate (say on slice, food, etc.), the moldy mass called mycelium, is visible to naked eye. > Molds have considerable value: used to produce antibiotic penicillin; soy sauce, cheeses and many other products, biosorption > Responsible for deterioration of materials such as textiles, woods, etc., Cause diseases in animals, plants and humans, (Peanuts spoilage).
- Yeasts are unicellular fungi > Shapes: Spherical to ovoid; ellipsoidal to filamentous > Both beneficial and detrimental: Used in baking industry (produce gas and makes dough rise), alcohol production, spoil food and cause diseases.

Bacteria

- Procryotes > Eubacteria and archaeobacteria (Based on ribosomal RNA differences)
- Eubacteria: Unicellular; Variety of shapes; 0.5 to 5 µm; often appear in pairs, chains, tetrads (group of four) or clusters; Essential in recycling wastes and production of antibiotics such as streptomycin; Cause diseases > sore throat, tetanus, plague, cholera, tuberculosis.
- Archaeobacteria: Have ability to survive in unusually harsh environments (high temperature, acids, salts, etc.); Some are capable of unique chemical activity > e.g. production of CH_4 from CO_2 & H_2 (live in environments with no oxygen, such as deep in swamp mud, or in the intestines of ruminants such as cattle and sheep).

Viruses

Border line between living and non living; not cells (much smaller 20 to 300 nm); simple in structure; get into the genetic material and damage the cell; Cause variety of diseases such as AIDS (HIV), common cold; genital herpes; poliomyelitis; hepatitis, tobacco mosaic (disease of tobacco plant), etc.; Also implicated for growth of some malignant tumors; Contain only one type of nucleic acid RNA or DNA, surrounded by protein coat.

Microorganisms and the Environment

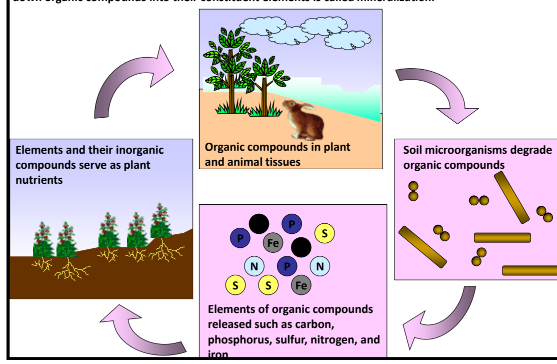
- Microorganisms are everywhere
  - Air current carries them to upper atmosphere and from continent to continent
  - Microorganisms inhabit all marine environments, from surface waters to the bottom of ocean trenches
  - There may be billions of them in a tea spoon of fertile soil
  - Total mass of microbial cell on the earth = 25 times the total mass of animal life
  - Animals carry large population of microbes on their body surfaces, in the intestinal track, and in their body openings.

Microorganisms and the Environment

- Human body contains 10 trillion cells and 100 trillion microorganisms
  - 10 microorganisms for each cell
  - Bacteria aid in digestion and account for 50% of weight of human and animal feces
  - Relatively few can cause disease; however, they have created an impression that all microorganisms are germs and are harmful.
- Microorganisms play a key role in recycling of elements in nature.

*The food chain, animal eats plants and other animals, and plant use animal wastes for nutrients; but microorganisms act as **translators** in this process.*

A schematic illustration of the role of microorganisms in the recycling of compounds and elements (natural resources) in nature. Elements bound in complex organic molecules are released by the metabolic activities of microorganisms and made available as plant nutrients. The process of breaking down organic compounds into their constituent elements is called *mineralization*.



Microbiology as a Science

- There are two major areas of study in the field of microbiology: basic microbiology, where the fundamental nature and properties of microorganisms are studied, and applied microbiology, where information learned from basic microbiology is employed to control and use microorganisms in beneficial ways.

Basic Microbiology

- Morphological characteristics: Shape, size, chemical composition and functions of internal structures.
- Physiological characteristics: Specific nutritional requirements and physical conditions needed for growth and reproduction.
- Biochemical activities: Degradation and synthesis reaction.
- Genetic characteristics: Inheritance and the variability of characteristics.
- Disease causing potential: Presence/absence for human, animals, plants, includes the study of host resistance to infection.
- Ecological characteristics: Natural occurrence and Interaction with others and environment.
- Classification: Taxonomic studies.

Applied Microbiology

Useful applications of microbiology are unlimited in their scope and variety

Medicine,
food (Single cell proteins, SCPs)
dairy products,
agriculture,
industry or the environment

Characterization of Microorganisms

- Under natural conditions microbial populations contain many different species
  - Not only of bacteria, but also of yeasts, molds, algae, protozoa, etc.
- Frequently it is important to identify how many and what kinds of MOs are present in a particular environment.
- We must have techniques to isolate, enumerate, and identify the microbes in a sample of material.

Pure Culture Techniques

- Isolation and cultivation of pure cultures.
- Preservation of pure cultures.
- 4 to 10 ^{o}C storage.
- May have to be transferred every day in new media.
- For long term storage, cultures are kept in tanks of liquid nitrogen (–196 ^{o}C), or in the freezers at –70 to –120 ^{o}C, or frozen and then dehydrated and sealed under vacuum in a process called lyophilization (freeze drying).

Identification of MOs

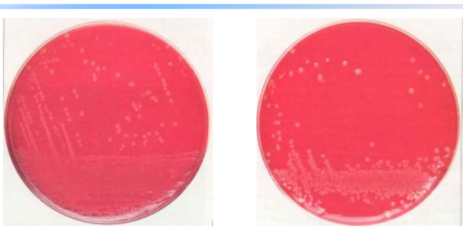
- Involve different techniques that may include the use of different media and different chemical reactions, but one of the powerful detective techniques is microscopic examination.

Microscopic Examination

- **Light or Optical Microscope**
  - 1000 or at the most 2000 times (resolving power; 0.25μm)
  - Bright field
    - → Uses a direct light source that illuminates the entire specimen field
    - → Since the microorganisms are transparent, they do not stand out distinctly with this type of microscopy
    - → Microbiologists usually stain, or color with dye, those microorganisms viewed with bright field microscopy.
  - Dark field
    - → Uses a light microscope, but brightly illuminates the microorganism against a dark background
    - → Looks like a dancer in a spotlight on a stage against a black curtain
  - Fluorescence microscopy
    - → Specimen is stained with a fluorescent dye
    - → Fluorescent antibody test.
  - Phase contrast microscopy
    - → Light microscopy that permits greater contrast

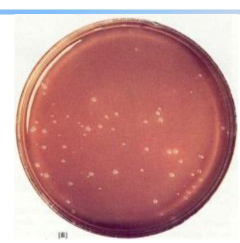
Colonies of microorganisms that have grown on a nutrient agar plate after being exposed to room air

Plate culture techniques for isolation of microorganisms in pure culture



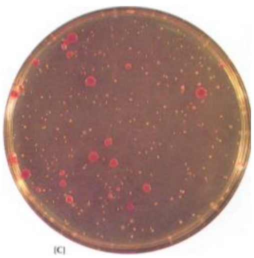
Streak-plate Method
The specimen is streaked onto the surface of the agar medium with a loop needle to thin out the population so that on some regions cells will later grow into isolated colonies

Plate culture techniques for isolation of microorganisms in pure culture



Spread-plate Method
A drop of diluted sample of the specimen is placed on the surface of an agar medium, and this drop is spread over the entire surface using a sterile bent glass rod.

Plate culture techniques for isolation of microorganisms in pure culture



Pour-plate method

The specimen, in this instance a culture of *Serratia marcescens,* is diluted by addition to tubes of melted (cooled) agar media. After through mixing, the tubes of inoculated media are poured into sterile Petri dishes; after solidification they are incubated. In this procedure colonies will grow both on and below the surface (subsurface colonies), since some cells are trapped within the agar medium when it solidifies.

In each of these techniques the objective is to thin out the microbial population so that individual cells are located at a distance from other cells. The individual cells, if far enough apart, will produce a colony that does not touch other colonies . All the cells in a single colony have the same parentage. To isolate a pure culture, a transfer is made from an individual colony onto a medium in a test tube.

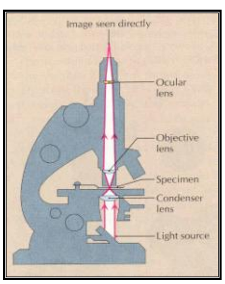
Identification of MOs

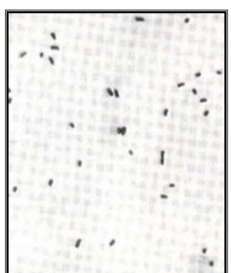
- Electron Microscope
  - Permits greater magnifications because of greater resolving power.
  - Beam wavelength in the range of 0.005 to 0.003 nm.
  - Resolving power 0.003µm as against 0.25µm in light microscopy
  - Scanning electron microscopy (SEM)
  - Transmission electron microscopy (TEM)

  A microscope's useful magnification is limited by its resolving power, or its ability to distinguish images of two close objects as separate, distinct entities. The resolving power is a function of the wavelength of light and the numerical aperture of the lens system. Light microscopes, by using visible light have a resolving power of approximately 0.25 µm

- Preparing Microorganisms for Light Microscopy
  - Wet-mount and Hanging-drop techniques
  - Staining technique: Simple staining, differential staining, gram staining.

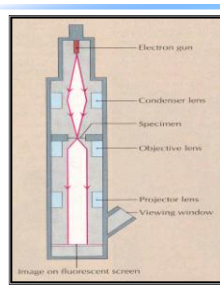
Diagrammatic Comparison of Imaging Systems Optical Microscope

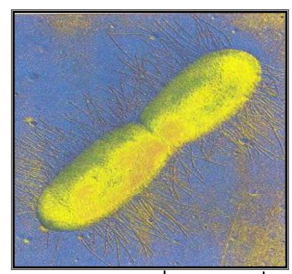




10 µm

Diagrammatic Comparison of Imaging Systems Transmission Electron Microscope (TEM)



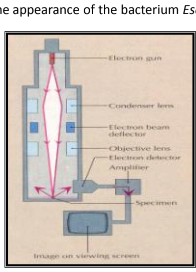


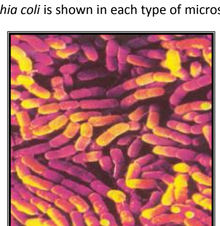
0.1 µm

Diagrammatic Comparison of Imaging Systems

Scanning Electron Microscope (SEM)

The appearance of the bacterium *Escherichia coli* is shown in each type of microscope





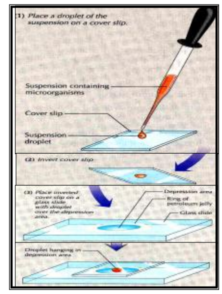
10 µm

A Comparison of Different Types of Microscopy

Type of microscopy	Maximum useful magnification	Appearance of specimen	Useful applications
Bright-field	1000-2000	Specimens stained or unstained; bacteria generally stained and appear color of stain	Gross morphological features of bacteria, yeasts, molds, algae, and protozoa
Dark-field	1000-2000	Generally unstained; appears bright or "lighted" in an otherwise dark field	Microorganisms that exhibit some characteristics morphological feature in the living state and in fluid suspension, e.g., spirochetes
Fluorescence	1000-2000	Bright and colored; color of the fluorescent dye	Diagnostic techniques where fluorescent dye fixed to organism reveals the organism's identity

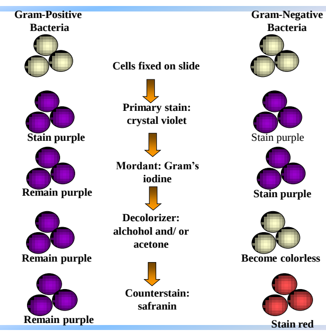
A Comparison of Different Types of Microscopy

Type of microscopy	Maximum useful magnification	Appearance of specimen	Useful applications
Phase-contrast	1000-2000	Varying degrees of "darkness"	Examination of cellular structures in living cells of the larger microorganisms, e.g., yeasts, algae, protozoa, and some bacteria
Electron	200,000-400,000	Viewed on fluorescent screen	Examination of viruses and the ultra-structure of microbial cells



Schematic illustration of the preparation of a hanging drop slide

The color appearance of bacterial cells at each step of the gram stain procedure.



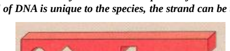
Information Used to Characterize Microorganisms

Microscopic to analysis of genetic material.

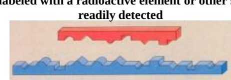
- Morphological characteristics: Shape, size, arrangement, and structure of both whole cells and internal components
- Nutritional and cultural characteristics
- Metabolic characteristics
- Antigenic characteristics
  - Antigen
  - Stimulates the production of antibodies
- Pathogenic characteristics
- Genetic characteristics

Schematic illustration of the principle of the DNA probe technique of the identification of bacteria

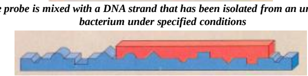
A piece (strand) of DNA is isolated from a "known" species of bacteria. If the nucleotide sequence of this strand of DNA is unique to the species, the strand can be used as a DNA probe



The DNA strand is labeled with a radioactive element or other substance that can be readily detected



The probe is mixed with a DNA strand that has been isolated from an unknown bacterium under specified conditions

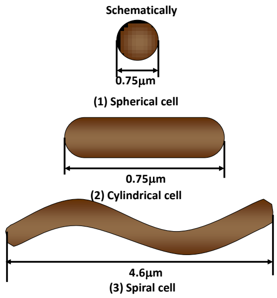


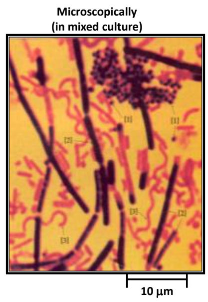
If the DNA probe and strand combine to form a duplex (double stranded DNA), the unknown bacterium is one of the same species as that from which the probe was isolated

Procaryotic Cell Structure

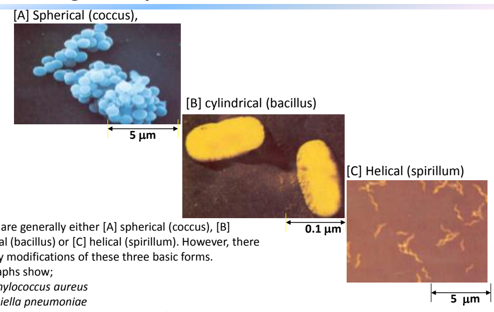
- **Gross Morphological Characteristics**
  - Size
    - 0.5 to 2 µm in diameter/width & 2-3 to 100 µm length
      - High Surface Area to Volume Ratio
  - Shape
    - Three basic shapes: spherical (*coccus/cocci*);
    - cylindrical or rod like (*bacillus/bacilli*);
    - spiral or helical (*spirillum/spirilla*)

Relative Sizes of three types of bacteria shown





Bacteria are generally either



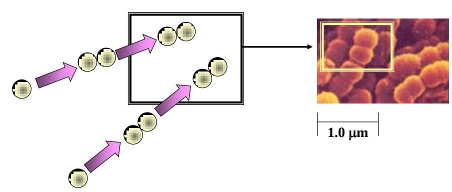
Bacteria are generally either [A] spherical (coccus), [B] cylindrical (bacillus) or [C] helical (spirillum). However, there are many modifications of these three basic forms.
Micrographs show;
[A] *Staphylococcus aureus*
[B] *Klebsiella pneumoniae*
[C] *Aquaspirillum itersonii (negative stain)*

Procaryotic Cell Structure

- **Arrangements**
  - Often attached to each other;
  - Grow in characteristic arrangement or patterns;
  - Cocci can grow in several arrangements depending on the plane of cellular division and daughter cells staying together following cell division;
    - Is typical of a particular species and can be used for identification;
  - *Diplococcus* (OO) and *Streptococcus* (in chain: OOOOOO);
    - divides in one plane and remains attached after several cell divisions;
  - Tetrads: *tetracoccus*, divide in two planes (groups of four);
  - Division in third plane can result in cubical packets of eight cells known as *sarcinae*;
  - Division in three planes in an irregular arrangement (grapelike cluster)
    - *Staphylococcus*;
  - Bacilli do not generally arrange themselves except a few; match sticks side by side (*diphtheria bacillus*), some in chains (*streptobacilli – Beggiatoa*).

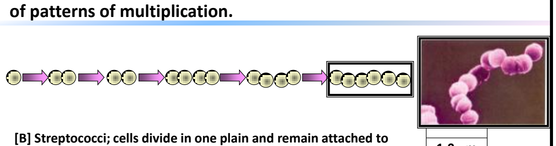
Together, the size, shape, and arrangement of bacteria constitute their gross morphology

Characteristics arrangements of cocci, with schematic illustrations of patterns of multiplication.

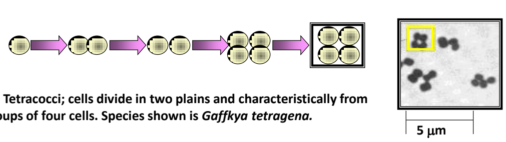


[A] Diplococci; cells divide in one plain and remain attached predominantly in pairs (scanning electron micrograph)

Characteristics arrangements of cocci, with schematic illustrations of patterns of multiplication.

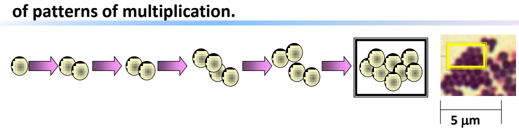


[B] Streptococci; cells divide in one plain and remain attached to form chains (scanning electron micrograph)

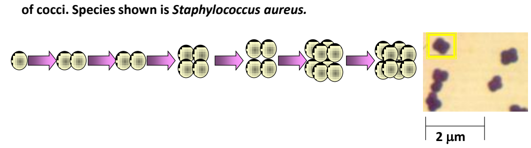


[C] Tetracocci; cells divide in two plains and characteristically from groups of four cells. Species shown is *Gaffkya tetragena.*

Characteristics arrangements of cocci, with schematic illustrations of patterns of multiplication.

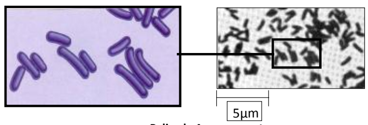


[D] Staphylococci; cells divide in three plains, in an irregular pattern, producing "bunches" of cocci. Species shown is *Staphylococcus aureus.*

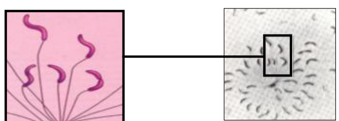


[E] Sarcinae; cells divide in three planes, in a regular pattern, producing a cuboidal arrangement of cells

Patterns of arrangements of Bacilli

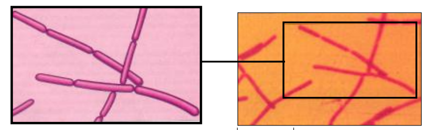


Palisade Arrangement



Rosette Arrangement

Patterns of arrangements of Bacilli



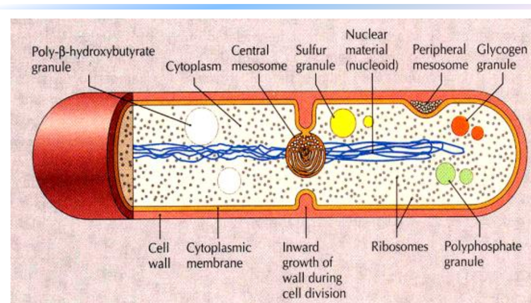
Streptobacilli Arrangement

Ultrastructure of Procaryotic Microorganisms

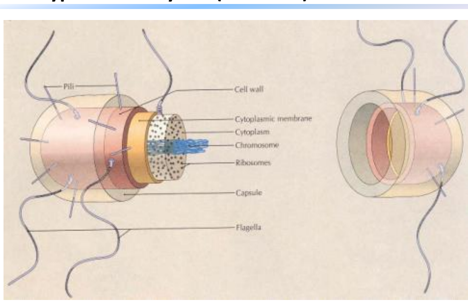
- A closer look at the individual cell structure (through microscope) gives a better idea of how they function. Some structures are outside, others are inside; Some are common (cell wall and cytoplasmic membrane), some others are present only in certain species or only under certain environmental conditions.

- **Flagella** (flagellum): → Three parts
  - A Basal body (a fine piece of engineering);
  - A short hook like structure; and a long helical filament; Help propel through liquid; 100µm/sec;
  - 3000 body lengths/min (cheetah fastest moving animal; 1500 body length/min)

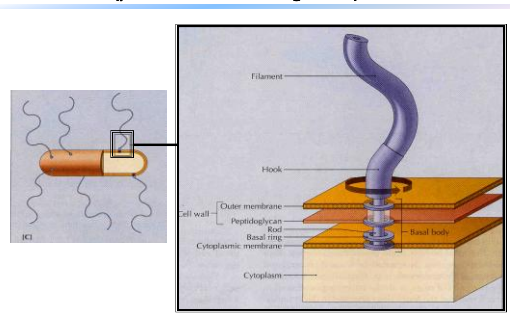
Major cell structure which occur within the bacterial cell. Certain structures, e.g., granuels or inclusions, are not common to all bacterial cells.



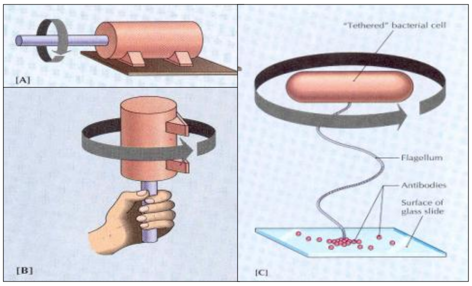
Diagrammatic Representation of the General Structure of a Typical Procaryotic (Bacterial) Cell



The mode of flagellar attachment to a Gram-negative bacterial cell (*pseudomonas aeruginosa*)



How Do You Know Bacterial Flagella Spin if you Can't See Them?



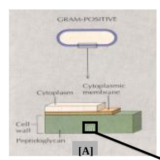
Ultrastructure of Procaryotic Microorganisms

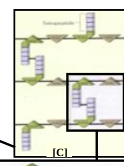
- **Pili (Pilus) or Fimbriae ((Fimbria):**
  - Appendages that have nothing to do with mobility
  - Hollow like flagella, but are nonhelical
  - Thinner, shorter and more numerous than flagella
  - Penetrate the cell wall but no complex anchoring system
  - Different types associated with different functions
  - F pilus involved in sexual reproduction of bacteria
  - Other types of pili help adhesion/attachment to surface (plathogenic bacteria)
- **Glycocalyse**
  - Layer of viscous material surrounding the cell;
  - Capsule if organized into a defined structure and attached firmly to the cell wall; or slime layer if disorganized, without any shape and attached loosely to the cell wall;
  - Usually made of polysaccharide;
  - Functions – Adherence, polar groups can protect against temporary drying by binding water molecules, serve as a reservoir of stored food.

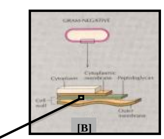
Ultrastructure of Procaryotic Microorganisms

- **Cell wall:**
  - Rigid structure that maintains the characteristic shape
  - Very high pressure or other severe physical conditions rarely change the shape
  - Prevents cell from expanding and eventually bursting because of water uptake
  - May account for 10 to 40 % of the dry weight of the cell
  - Not homogeneous, but in layers of different substances which differ in thickness and composition → induce some of the characteristic traits of bacteria such as Gram staining, disease causing ability, etc.
  - Serves as barrier to some substances – preventing
- **Cytoplasmic Membrane:**
  - Beneath the cell wall bilayer
  - Composed primarily of phospholipids (20 to 30 %) and proteins (50 to 70 %)
  - 7.5 nm thick
  - Barrier to most water soluble molecules and much more selective than the cell wall
  - Contain specific proteins called permeases

Peptidoglycan in the cell wall of bacteria

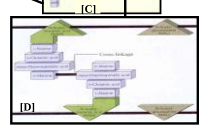




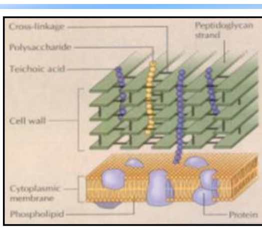


[A,B] location of peptidoglycan in Gram-positive and Gram-negative bacteria, respectively.

[C] the peptidoglycan polymer consist of repeating units of N-acetylglucosmine (NAG) linked to N-acetylmuramic acid (NAM) with a peptide side chain of four amino acids attached to NAM. Area within colored box is enlarged in [D]

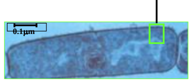


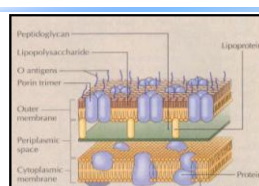
[D] This enlargement shows the basic building block of peptidoglycan in *Escherichia Coli*. NAM subunits of two neighbouring polysaccharide chains are directly cross linked by the peptide chains. Other species of bacteria may have interpeptide bridges, i.e., peptides linking the peptide chain from NAM



Diagrammatic representation of the differences between the fine structure of the Gram-positive cell wall and the Gram Negative cell wall of bacteria.

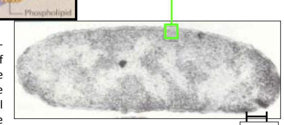
Structure of the cell wall of a Gram - positive Bacterium (*Bacillus sp.)*



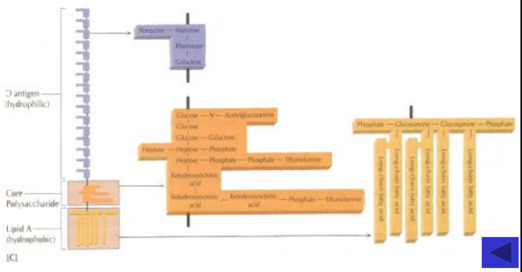


Diagrammatic representation of the differences between the fine structure of the Gram-positive cell wall and the Gram Negative cell wall of bacteria.

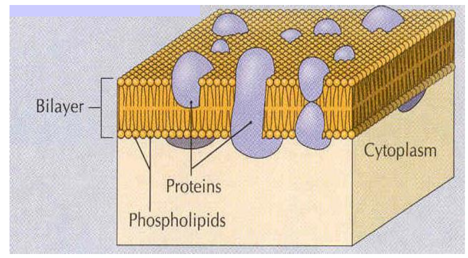
Structure of the Cell wall of a Gram-Negative bacterium. Electron Micrograph of a stained thin selection of the marine bacterium *Alteromonas haloplankits.* The organism has the simple ultrastructural characteristics of a typical Gram-negative bacterium.



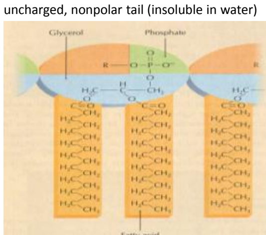
Structure of one unit of *Salmonella* cell wall lipopolysaccharide (LPS). This structure may vary slightly from one genus of Gram-negative bacterium to another. However, all the cell-wall LPSs contain the three general regions shown: Lipid A, Core Polysaccharide, and O antigen (which extends into the surrounding medium).



Phospholipids are arranged in a bilayer such that the polar portions (spheres) face outward and the nonpolar portions (filaments) face inward. Protien components are shown as circumscribed solids.



Example of a eubacterial phospholipid, showing two unbranched long-chain fatty acids esterified to glycerol. (R is any of several compounds such as ethanolamine, chlorine, serine, inositol, or glycerol) The phosphate end is the charged, polar head (soluble in water), while the hydrocarbon end is the uncharged, nonpolar tail (insoluble in water)



Ultrastructure of Procaryotic Microorganisms

- **Internal cell structures:**
  - Cytoplasmic area containing dissolved substances and particles such as ribosones, etc.
  - Nuclear material or nucleoid.
- **Dormant forms:**
  - These resting forms are metabolically inactive (non-growing)
  - Can germinate (begin to grow) under appropriate conditions
  - Can survive unfavourable conditions.
- **Spores**
  - Endospores- spores that form within the cell
  - Actinomycetes
  - Cysts - developed from vegetative cells; Less resistant.

Dormant forms of Prokaryotic Microorganisms

- Some species of bacteria produce *dormant* forms called ***spores*** and ***cysts*** that can survive unfavourable conditions, such as drying or heat.
- These resting forms are metabolically inactive, which means that they are not growing.
- However, under appropriate environmental conditions, they can germinate (begin to grow) and become metabolically active *vegetative* cells, which grow and multiply.

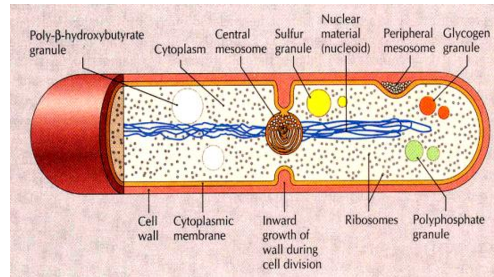
Spores

- Spores that form within cell, called ***endospores,*** are unique to bacteria. They are thick-walled, highly refractile (very bright with light microscopy), and highly resistant to environmental changes.
- It is necessary to use heat when staining an endospore for light microscopy, to make the spore absorb the dye. Produced one per cell, endospores vary in shape and location within the cell.
- When endospores are freed from the mother cell, or ***sporangium,*** they can survive extreme heat, drying, exposure to toxic chemicals such as some disinfectants.

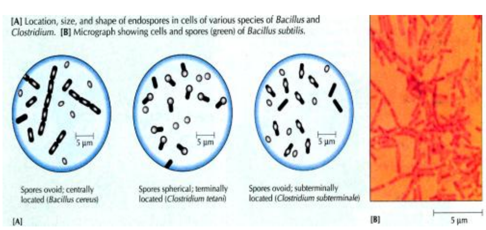
Spores

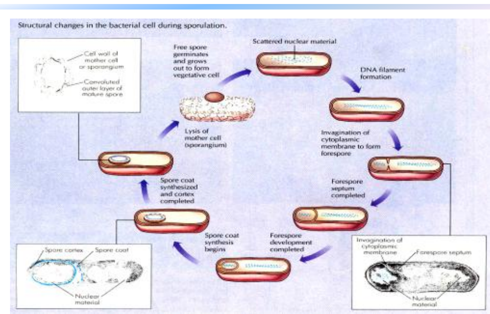
- The structural changes that occur during the development of endospores have been extensively studied.
- Under the right conditions, a spore will form a vegitative cell.
- This germination may be triggered by brief exposure to heat or by mechanical forces acting on the spore.

Major cell structure which occur within the bacterial cell. Certain structures, e.g., granuels or inclusions, are not common to all bacterial cells.



[A] Location, size, and shape of endospores in cells of various species of *Bacillus* and *Clostridium*
[B] Micrograph showing cells and spores (green) of *Bacillus subtilis*





Cysts

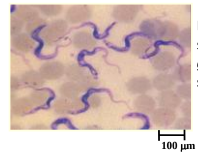
- Like endospores, cysts are dormant, thick-walled forms that resist drying. They develop from a vegetative cell and can later germinate under suitable conditions
- However, the structure and chemical composition are different from that of endospores, and they do not have the high heat resistance.

Morphological Characteristics of Eucaryotic Microorganisms

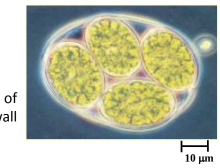
- All prokaryotic organisms are microorganisms while only a few groups of eucaryotic organisms include microorganisms.
- These groups – algae, fungi, and protozoa – include a vast diversity of organisms. Among them are species too large to be considered microscopic (examples: seaweeds which are algae, and the mushrooms which are fungi).
- They are related to the microscopic eucaryotes and are usually included within the scope of microbiology.
- The morphological differences among fungi, protozoa and algae results in dramatic structural diversity among microorganisms.

A mold *Aspergillus niger* as seen by scanning electron microscopy. Note the aerial filaments bearing large heads of spores called conidia.

A budding yeast as seen by scanning electron microscopy



Light photomicrograph of the sleeping sickness protozoan *Trypanosoma gambinese.* The flagelled protozoan is seen among the red blood cells



Oocystis, a green alga, occuring as a group of cells surrounded by a cell wall

Morphology of Fungi

- Yeasts and molds are fungi, but they differ in their morphology.
- Yeast Cells: Generally larger than most bacteria, 1 to 5 μm in width and 5 to 30 μm or more in length; Commonly oval but some are elongated or spherical; Each species has a characteristic shape, but even in a pure culture individuals vary considerably in shape and size; Lack flagella and other means of locomotion; On agar medium they form smooth, glistering colonies that resemble those of bacteria.
- Molds: Multicellular that look like filaments under low magnification and with high magnification can look like tiny jungles with many parts; The body consists of mycelium and the dormant spores; Each mycelium is a mass of filaments called hyphae; Each hypha is about 5 to 10 μm in width and formed by joining together of many cells; The rigid walls of hyphae are made of chitins, celluloses, and glucans.

Morphology of Algae

- Mixture of variety of sizes and shapes; Species range from single microscopic cells to organisms hundreds of feet long; Single celled species may be spherical, rod-shaped, club-shaped, or spindle-shaped; Multicellular ones appear in a variety of forms and degrees of complexity – filaments in series or intertwine, in colonies with simple aggregations of single cells or different cell types with special functions.

Morphology of Protozoa

- Oval, spherical, elongated or polymorphic;
- Morphologically different forms at different stages of life cycle;
- Size: 1 to 2000 μm; Lack cell walls;
- Able to move at some stage of their life cycle;
- Ingest food particles; Individual cell is a complete organism; Some form cysts.

Ultra Structure of Eucaryotic Microorganisms

- Cells are generally larger and structurally more complex; Have membrane bounded nucleus; Morphology can include appendages, cell walls, membranes, and various internal structures.

- **Flagella and Cilia:** Thin structure used for locomotion; Originate from a basal body lying beneath the cell membrane; Cilia identical to flagella in structure but are shorter and more numerous; Cell covered with cilia looks like a porcupine; Cilia have coordinated rhythmic motion as against whipping motion of flagella; Movement powered by hydrolysis of ATP unlike protonmotive force in prokaryotes.
- **Cell Wall:** Differ in chemical composition: peptidoglycan in prokaryotes, polysaccharides such as cellulose and pectin in plant cells, chitin and cellulose in fungi, varying amounts of cellulose, other polysaccharides, and calcium carbonate in algae.

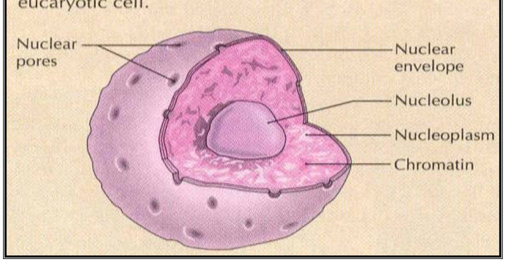
Ultra Structure of Eucaryotic Microorganisms

- **Cytoplasmic Membrane:** Semipermeable; Lipid bilayer with inserted proteins that may protrude on one side or the other; Morphologically and functionally resembles that of prokaryotes; Differences – Contains sterols to add strength; No enzymes involved in metabolic energy generation.
- **Cellular organelles:** Protoplasm – karyoplasms and cytoplasm; Nucleus, endoplasmic reticulum, golgi complex, mitochondria, chloroplasts.

Ultra Structure of Eucaryotic Microorganisms

- **Nucleus:** Double membrane envelope; Numerous large pores to allow passage of RNA and proteins; Give rise to or continuous with endoplasmic reticulum; Spherical or oval; Largest organelle contains cell's hereditary information in the form of DNA; In the non dividing karyoplasms, DNA combines with proteins that gives febrillar appearance, called chromatin, and during cell division chromatin condenses into chromosomes; Nucleolus, dense within karyoplasms, about 5 to 10% of nucleolus is RNA and rest is protein, site of synthesis of rRNA, ribosome - subunits are protein and RNA, formed in nucleus and fully functional cytoplasm.

Diagrammatic representation of the nucleus of a eucaryotic cell.



Ultra Structure of Eucaryotic Microorganisms

- **Endoplasmic Reticulum (ER):** Is a membranous network; Two types - rough and smooth; Rough is studded with ribosomes; Involved in protein synthesis; Smooth involved in synthesis of glycogen, lipid and steroid; Connected to nuclear and cytoplasmic membranes.
- **Golgi complex:** Looks similar to smooth ER but with vesicles at their lips; Connected to cell's cytoplasmic membrane; Packaging center responsible for safe transport of synthesized compounds; Also acts as distribution center for cell.
- **Mitochondria:** Singular - Mitochondrion; Site for ATP generation in aerobic cells (powerhouse) i.e. site for electron transport (cell wall for bacterial cells).
- **Chloroplasts:** Another energy generating cytoplasmic organelle in algae, site for photosynthesis.

Schematic diagram of the general structure of a typical eucaryotic (animal) cell.

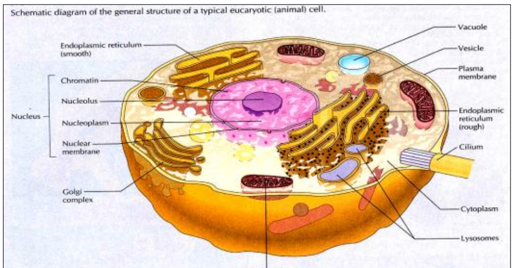
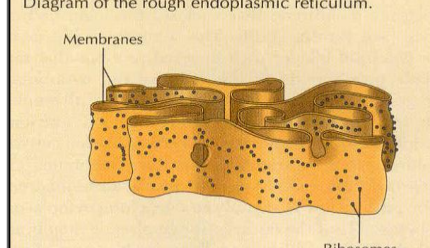
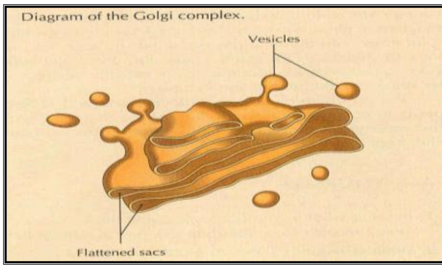
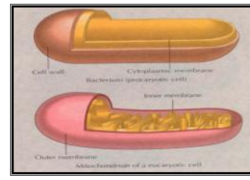


Diagram of the rough endoplasmic reticulum.

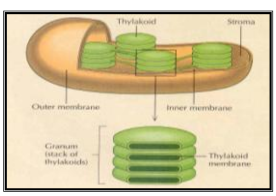




In bacterial cells the electron-transport system occurs in the cytoplasmic membrane. In eucaryotic cells the electron-transport system is located within small organelles called *mitochondria*, which are about 1 to 3 μm long (the same size as many bacteria). A mitochondrion has two membranes; the inner one contains the electron -transport system and has numerous infoldings



In eucaryotic cells, thylakoids occur within special organelles called *chloroplast*, which are larger than mitochondria. In the chloroplast of plant cells the thylakoids are flattened, disk-shaped sacs arranged in stacks; each stack is called a *granum* (plural, *grana*). Some of the granum thylakoids are connected to thylakoids in other grana.



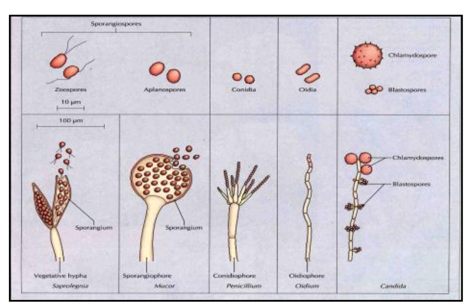
Dormant Forms of Eucaryotic Microorganisms

- As discussed earlier, some microorganisms can produce dormant forms called spores and cysts that can withstand unfavourable conditions.
- Both fungi and protozoa include species that use such resting structures for protection and reproduction.
- Algae also form spores but their main function is for reproduction. Algae do not form cysts.

Dormant forms of Eucaryotic Microorganisms

- Spores
  - Fungi produce both *sexual* and *asexual* spores. Sexual spores are produced as a result of the fusion of two specialized reproductive cells called *gametes* into one fertilized cell. The formation of sexual spores does not involve the fusion of gametes.

Types of asexual spores in the fungi.



Dormant forms of Eucaryotic Microorganisms

- Cysts
  - Many protozoa produce resting forms called *cysts.*
  - There are two possible forms of protozoan cysts:
    - *Protective cysts*
    - *Reproductive cysts*
  - The vegetative forms of protozoa, or ***trophozoites***, synthesize protective cysts that are resistant to drying, lack of food, lack of oxygen, or acidity in hosts stomach.
  - By contrast, reproductive cysts are not induced by adverse environmental conditions. They are often thin-walled and lack of resistance of protective cysts.

Dormant forms of Eucaryotic Microorganisms

- **Cysts**
  - Parasitic species of protozoa often move from host to host as cysts, making these structures important as modes of transmission as well. Such cysts from in the intestinal tract and are excreted in feces, which contaminate water and food ingested by the next host.
  - With many of these parasites, the cysts is the only way the protozoan is able to survive outside the host.

Nutritional Requirements & Microbiological Media

- Off all living organisms, microorganisms are the most versatile and diversified in their nutritional requirements.
- All living organisms share some common nutritional needs, like the need of carbon, nitrogen and water.
- Water is particularly important to microorganisms, because most microorganisms can absorb nutrients only when the chemicals are dissolved in water.
- These chemical requirements, along with the physical requirements, must be satisfied by the organism's environment in order for it to grow.
- In order to characterize the morphological, physiological, and biochemical properties of microbes, laboratory cultivation of microbial cell is necessary.

Nutritional Requirements & Microbiological Media

- Controlling the environment in which microorganisms are growing can be used to make accurate identification of species and measurements of microbial growth.
- The cultivation of microorganisms requires the appropriate culture *media* (singular medium).
- Media are nutrient preparations used for growth of microbes in laboratory.
- Many microbes, as well as cells of both plants and animals, can be grown in vitro (in laboratory vessels) if proper media is used. Some microorganisms, for e.g., the causative agent of leprosy – must be grown in vivo, or in a living host organism.

Chemical Elements as Nutrients

- In order to grow, all organisms need a verity of chemical elements as nutrients. These elements are necessary for both the synthesis and normal functions of cellular components.
- The main elements for cell growth include carbon, nitrogen, hydrogen, oxygen, sulfur and phosphorus.

Carbon

- Those microbes that use organic compounds as their major carbon source are called ***heterotrophs.***
- Microorganisms that use carbon di oxide (the most oxidized form of carbon) as their major or even sole source of carbon are called ***autotrophs.***

Nitrogen

Hydrogen, Oxygen, Sulfur, and Phosphorus

- Other elements essential to all organisms are hydrogen, oxygen, sulfur and phosphorus.
- Hydrogen and oxygen form many organic compounds. Sulfur is needed for biosynthesis of the amino acids cysteine, cystine, and methionine.
- Phosphorus is essential for the synthesis of nucleic acids and ***adenosine triphosphate (ATP),*** a compound that is extremely important for energy storage and transfer.

Other Elements

Nutritional Classification of Microorganisms

Organisms that use chemical compounds for energy are called ***chemotrophs.*** Those that depend primarily on radiant energy (light) are designated ***phototrophs.*** By combining these terms with those concerning principal carbon sources, the following groupings emerge:

- ***Chemoautotrophs*** – those organisms that use chemical substances (inorganic) as sources of energy and carbon di oxide as the main source of carbon.
- ***Chemoheterotrophs*** – those that use chemical substances (organic) as sources of energy and organic compounds as the main source of carbon
- ***Photoautotrophs*** – those that use light as a source of energy and carbon di oxide as the main source of carbon.
- ***Photoheterotrophs*** – those that use light as a source of energy and organic compounds as the main source of carbon

Nutritional Classification of Bacteria and Other Organisms

Nutritional group	Carbon Source	Energy Source	Examples
Chemoautotrophs	Carbon di Oxide	Inorganic Compounds	Nitrifying, hydrogen, iron, and sulfur bacteria
Chemoheterotrophs	Organic Compounds	Organic Compounds	Most bacteria, fungi, protozoa, and animals
Photoautotrophs	Carbon dioxide	Light	Purple sulfur and green sulfur bacteria, algae, cynobacteria, and plants
Photoheterotrophs	Organic Compounds	Light	Purple nonsulfur and green nonsulfur bacteria

Media Used for Cultivating Microorganisms

- Microbiologists normally add ***agar,*** a complex polysaccharide extracted from marine algae.
- Agar, which is used at a concentration of about 1.5 % (w/v) or 15 g/l, has several properties that make it the ideal solidifying agent.
- Agar becomes a transparent molten solution at about the boiling point of water (100ºC) and then remains liquid down to about 40ºC. Because most microorganisms are not killed at 45ºC, they can be added to a liquid medium containing molten agar before the medium is poured into Petri plates/dishes.
- Agar is not a nutrient for most microorganisms and is not metabolized during microbial growth.

Media for the Growth of Bacteria

Chemically Defined Medium for a Chemoautotrophic Bacterium

Ingredient	Function	Amount
$(NH_4)_2SO_4$	Nitrogen as well as energy Source	0.5 g
$NaHCO_3$	Carbon source in the form of CO_2 in aqueous Solution	0.5 g
Na_2HPO_4	Buffer and Essential ions	13.5 g
KH_2PO_4	Buffer and Essential ions	0.7 g
$MgSO_4.7H_2O$	Essential ions	0.1 g
$FeCl_3.6H_2O$	Essential ions	0.014 g
$CaCl_2.2H_2O$	Essential ions	0.18 g
Water	Solvent	1000 ml

Media for the Growth of Bacteria

Chemically Defined Medium for a Heterotrophic Bacterium

Ingredient	Function	Amount
Glucose	Carbon and energy source	1 g
$NH_4H_2PO_4$	Nitrogen source, buffer, and essential ions	5 g
K_2HPO_4	Buffer and essential ions	1 g
NaCl	Essential ions	5 g
$MgSO_4.7H_2O$	Essential ions	0.2 g
Water	Solvent	1000 ml

The above ingredients represent the minimum constituents in a medium for a non fastidious bacterium such as wild-type *Eschericha coli*. For a fastidious species, such as *Lactobacillus acidophilus*, additional substances such as amino acids and vitamins have to be added to the medium.

Media for the Growth of Bacteria

Composition of Nutrient Broth, a Complex Medium for the growth of Heterotrophic Bacteria

Ingredient	Function	Amount
Beef extract	Water-soluble substances of animal tissue: carbohydrates, organic nitrogen compounds, vitamins, salts	3 g
Peptone	Organic nitrogen, some vitamins	5 g
Sodium chloride	Ions and osmotic requirements	8 g
Water	Solvent	1000 ml

If a solid medium is required, agar (15 g) is added; the medium is called *nutrient agar.*

Media for the Growth of Bacteria

Some Commercially Available Selective and/ or Differential Media for Bacteria

Medium	Intended use	Principles of Use
MacConkey agar	Differential and selective medium for isolation and differentiation of Gram-negative lactose-fermenting bacteria from non-lactose-fermenting enteric bacteria	Colonies of bacteria able to ferment lactose produce a localized pH drop, which followed by absorption of neutral red indicator, imparts a red color to the colonies as well as a zone of precipitated bile. Bile salts also inhibit Gram-positive bacteria. Non-lactose-fermenting colonies remain colorless and translucent.
Deoxycholate agar	Differential and selective plating medium for the Gram-negative enteric bacilli	Coliform colonies (lactose-fermenting) are red; non-lactose-fermenting colonies are colorless. Sodium deoxycholate suppresses Gram-positive bacteria.

Media for the Growth of Bacteria

Some Commercially Available Selective and/ or Differential Media for Bacteria

Medium	Intended use	Principles of Use
Phenylethanol agar	Selective medium for the isolation of Gram-positive staphylococci and streptococci from specimen also containing Gram-negative organisms	Phenylethanol permits the growth of Gram-positive organisms but inhibits the growth of Gram-negative organisms found in the same specimen.
Columbia CNA agar	Selective medium to which blood is added, used for the isolation of Gram-positive cocci	The antimicrobial agents colistin and nalidixic acid in the medium supress growth of Gram-negative bacteria

Media for the Growth of Fungi

Composition of a General-Purpose Medium, Sabouraud's Agar, for the Isolation and Growth of Fungi

Ingredient	Function	Amount
Peptone	Source of carbon, nitrogen, elements	10 g
Glucose	Carbon and energy source; high concentration favors growth of fungi but inhibits growth of bacteria	40 g
Agar	Solidifying agent	15 g
Water	Solvent	1000 ml
pH	Low pH suppresses bacterial growth but enhances fungal growth	5.6

Media for Other Microorganisms

- Protozoa
- Algae

Various Types of Media

- Special-Purpose Media
- Selective Media
  - Selective Media are designed to enhance to growth of particular kind of microorganisms or suppress the growth of other kinds of microorganisms (some may do both).
- Differential Media
  - Microbiologists use differential media when they want to differentiate among various kinds of microorganism on an agar plate.
- Selective Differential media
  - Some culture media are both selective and differential.
- Enrichment Media
  - Natural environments are usually populated by numerous kinds of bacteria or other microorganisms. When a species of special interest is present, but only in very small numbers, microbiologist use an enrichment medium. The medium favors the growth of that species, but not the growth of the others present in the mixed population. Enrichment techniques provide an environment, both chemical and physical, that results in increased numbers of an initially scarce species. Unlike a selective medium, no inhibitory agent is used to prevent the growth of unwanted microorganisms.

Various Types of Media

- Microbiological Assay Media
  - Specific microorganisms can be used to measure the concentrations of substances such as antibiotics and vitamins. For instance, blood serum or other tissue fluids can be assayed for antibiotics by using microorganisms known to be susceptible to those antibiotics.

- Tissue Culture Media and Methods
  - Tissue cultures are plant or animal cells grown in the laboratory in specialized media. Tissue culture methods were developed to cultivate viruses in vitro, since viruses can replicate only inside living host cells.